

CLAIMS

1. A method for detection and characterization of *Mycobacterium tuberculosis* present in a sample, comprising the steps of:

- (a) obtaining a sputum sample suspected of containing *M. tuberculosis*,  
(b) performing a first sequencing procedure, with or without prior amplification, on the sample, said sequencing procedure generating sequencing fragments for evaluation of the rpoB, katG, rpsL/s12 and 23S genes for the presence of antibiotic-resistance inducing mutations when *M. tuberculosis* is present in the sample, wherein primers for the sequencing of the rpoB gene are selected such that the generation of sequencing products for this gene is indicative of the presence of *M. tuberculosis* in the sample; and  
(c) if *M. tuberculosis* is detected as a result of generation of sequencing products for the rpoB gene in step (b), performing a second sequencing procedure, with or without prior amplification, on the sample to evaluate at least one additional *M. tuberculosis* gene for the presence of antibiotic-resistance inducing mutations.

2. The method of claim 1, wherein the second sequencing procedure evaluates PR, embB pncA and gyrA genes for the presence of antibiotic-resistance mutations.

3. The method of claim 1, further comprising the step of performing a third sequencing procedure when *M. tuberculosis* was detected in step (b), separate from the first and second sequencing procedures, to evaluate 16S/rrs and mabA genes for the presence of antibiotic-resistance mutations.

4. The method of any of claims 1 to 3, wherein the first sequencing procedure for rpoB is performed using amplification primers as set forth in Seq. ID Nos. 1 and 2 and sequencing primers as set forth in Seq. ID. Nos. 3 and 4.

5. The method of any of claims 1 to 4, wherein the first sequencing procedure for katG is performed using amplification primers as set forth in Seq. ID Nos. 6 and 7 and sequencing primers as set forth in Seq. ID. Nos. 8 and 9.

a 6. The method of any of claims ~~1 to 5~~, wherein the first sequencing procedure for rpsL/s12 is performed using amplification primers as set forth in Seq. ID Nos. 21 and 22 and sequencing primers as set forth in Seq. ID. Nos. 23 and 24.

a 7. The method of any of claims ~~1 to 6~~, wherein the second sequencing procedure for 23S is performed using amplification primers as set forth in Seq. ID Nos. 46 and 47 and sequencing primers as set forth in Seq. ID. Nos. 48 and 49.

a 8. The method of any of claims ~~1 to 7~~, wherein the second sequencing procedure for PR is performed using amplification primers as set forth in Seq. ID Nos. 11 and 12 and sequencing primers as set forth in Seq. ID. Nos. 13 and 14.

a 9. The method of any of claims ~~1 to 8~~, wherein the second sequencing procedure for pncA is performed using amplification primers as set forth in Seq. ID Nos. 36 and 37 and sequencing primers as set forth in Seq. ID. Nos. 38 and 39.

a 10. The method of any of claims ~~1 to 9~~, wherein the second sequencing procedure for embB is performed using amplification primers as set forth in Seq. ID Nos. 31 and 32 and sequencing primers as set forth in Seq. ID. Nos. 33 and 34.

a 11. The method of any of claims ~~1 to 10~~, wherein the second sequencing procedure for gyrA is performed using amplification primers as set forth in Seq. ID Nos. 41 and 42 and sequencing primers as set forth in Seq. ID. Nos. 43 and 44.

a 12. The method of any of claims ~~2 to 11~~, wherein the third sequencing procedure for 16S/rrs is performed using amplification primers as set forth in Seq. ID Nos. 26 and 27 and sequencing primers as set forth in Seq. ID. Nos. 28 and 29.

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13. The method of any of claims 2 to 12, wherein the third sequencing procedure for *mabA* is performed using amplification primers as set forth in Seq. ID Nos. 16 and 17 and sequencing primers as set forth in Seq. ID Nos. 18 and 19.

14. A kit for evaluation of antibiotic-resistance mutations in a sample of *Mycobacterium tuberculosis*, comprising pairs of amplification primers and matched pairs of sequencing primers for amplification and sequencing the at least the *rpoB*, *katG*, *rpsL/s12* and *23S* genes of *M. tuberculosis*, characterized in that the amplification and sequencing primer pairs include at least one combination of primer pairs selected from among:

- (a) amplification primers of Seq. ID Nos. 1 and 2 in combination and sequencing primers of Seq. ID Nos. 3 and 4;
- (b) amplification primers of Seq. ID Nos. 6 and 7 in combination and sequencing primers of Seq. ID Nos. 8 and 9;
- (c) amplification primers of Seq. ID Nos. 11 and 12 in combination and sequencing primers of Seq. ID Nos. 13 and 14;
- (d) amplification primers of Seq. ID Nos. 16 and 17 in combination and sequencing primers of Seq. ID Nos. 18 and 19;
- (e) amplification primers of Seq. ID Nos. 21 and 22 in combination and sequencing primers of Seq. ID Nos. 23 and 24;
- (f) amplification primers of Seq. ID Nos. 26 and 27 in combination and sequencing primers of Seq. ID Nos. 28 and 29;
- (g) amplification primers of Seq. ID Nos. 31 and 32 in combination and sequencing primers of Seq. ID Nos. 33 and 34;
- (h) amplification primers of Seq. ID Nos. 36 and 37 in combination and sequencing primers of Seq. ID Nos. 38 and 39;
- (i) amplification primers of Seq. ID Nos. 41 and 42 in combination and sequencing primers of Seq. ID Nos. 43 and 44; and
- (j) amplification primers of Seq. ID Nos. 46 and 47 in combination and sequencing primers of Seq. ID Nos. 48 and 49.

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